

# DNA plasmids and cell lines

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 An abbreviated version of this protocol was published in Science in Jan 2020

Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles

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## Detailed protocol

Dear Shuhao,

Attached are the sequences of RA-Sec61B and GB-C1 (there was no GFP encoded here) plasmids.

RA-Sec61B - The sequence regions encoding for RA tag and Sec61B are 597-1289bp and 1311-1601bp, respectively.

1. The RA tag was PCR amplified from Addgene plasmid #61019 with overhangs encoding for NheI and BspEI cut sites.
2. The Clontech vector plasmid pAcGFP1-C1 was sequentially digested using NheI and BspEI restriction enzymes to remove GFP encoding region.
3. RA tag was ligated into the digested pAcGFP1-C1 vector to create RA-C1 vector.
4. Sec61B was PCR amplified from Addgene plasmid #49155 with overhangs encoding for XhoI and KpnI cut sites.
5. Sec61B was ligated into XhoI and KpnI digested RA-C1 to create RA-Sec61B.

GB-C1 empty vector - The sequence regions encoding for GB tag are 597-1274bp with the multiple cloning site encoded by 1275-1446bp.

1. The GB tag was PCR amplified from Addgene plasmid #61017 with overhangs encoding for NheI and BspEI cut sites.
2. The Clontech vector plasmid pAcGFP1-C1 was sequentially digested using NheI and BspEI restriction enzymes to remove GFP encoding region.
3. GB tag was ligated into the digested pAcGFP1-C1 vector to create GB-C1 vector.

Best,  
Jason

## Related files

 Lee et al sequences.docx



**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Lee, J. and Voeltz, G. (2020). DNA plasmids and cell lines. Bio-protocol Preprint. [bio-protocol.org/prep317](https://bio-protocol.org/prep317).
2. Lee, J. E., Cathey, P. I., Wu, H., Parker, R. and Voeltz, G. K. (2020). Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. Science 367(6477). DOI: [10.1126/science.aay7108](https://doi.org/10.1126/science.aay7108)

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